In vitro degradability and gas production parameters of Sericea lespedeza (*Lespedeza cuneata*) mixed with varying types and levels of roughages

J.O. Ouda[#], I.V. Nsahlai and P.M. Mahundu
Animal & Poultry Science, Faculty of Science & Agriculture, Private Bag X01, Scottsville 3209, South Africa

Abstract

Sericea lespedeza (*Lespedeza cuneata*) regrowth was harvested at early (ELP) or late (LLP) flowering stages, mixed with varying types and levels of roughage and fermented for 72 h *in vitro*, using the gas production (GP) technique. The roughage : lespedeza ratios were 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100. The roughages included maize stover harvested at grain milk (MM) or dry (MD) stages and veld grass hay (GH). The crude protein (CP) content of ELP, LLP, MM, MD and GH were 187, 97, 48, 29 and 34 g/kg dry matter (DM), respectively. The corresponding NDF values were 283, 589, 696, 73.3 and 665 g/kg DM. Degradability was slightly higher in MM as compared to MD and GH (means 704.9 *vs*. 676.6 and 685.0 g/kg, respectively) between ELP rations. The roughages had similar but lower degradability in LLP rations (means 633.4, 632.6 and 631.1 g/kg for MM, MD and GH, respectively). Increased proportion of ELP and LLP resulted in decreased degradability in all the roughages. Microbial yield was similar among roughages in ELP rations, but the roughages differed in microbial yield among LLP rations whereby GH had the highest value and MD the lowest. Increased proportion of ELP elicited an increase in microbial yield but increasing LLP had no effect (range 135.0 – 264.8 g/kg among ELP and 143.4 – 295. 9 g/kg among LLP rations). Roughage type affected GP with MD and GH having the lowest and highest values, respectively. The values ranged from 167.4 – 209.8 and 160.4 – 221.0 mL among ELP and LLP rations, respectively. There was a decrease in GP as the proportion of ELP or LLP increased and roughage type x supplement level interaction had effect. The ratio of degradability to GP, i.e. the partitioning factor (PF) ranged from 3.43 – 4.74 and 3.13 – 4.23 among ELP and LLP rations, respectively, whereby GH had highest and MD lowest values. The rate of GP from soluble fraction was not affected, but that of the fibre fraction differed among the roughages in ELP rations whereby GH had lower rate than MM and MD (mean 0.023 *vs*. 0.026 and 0.025, respectively). The lag time (lt) tended to be reduced as ratio of ELP increased (range 1.83 to 6.59 h). In LLP rations, roughage type, supplementation level, roughage type x supplementation level interactions affected lt. The GH had the longest and MM had the shortest lt among the roughages (range 0.88 – 9.61 h), and likewise lt reduced as ratio of LLP increased. The results indicate that the various nutritive attributes considered are differentially influenced by lespedeza type and level, roughage type and the interactions among these aspects, hence the importance of their implications in formulating ruminant diets. The results indicate that when using roughages with similar quality as those studied, lespedeza ratio of 40 - 60% of DM consumed can be beneficial.

Keywords: Lespedeza rations, fermentative activity, nutritive value, ruminants
[#] Corresponding author. E-mail: jackouda@hotmail.com, 204513911@ukzn.ac.za

Introduction

Feed resources are a major component of economic animal production. Their availability and efficiency of use in specific agro-ecological zones dictate to a very large extent the performance of the livestock production system. During the dry season ruminants in tropical and sub-tropical regions mostly survive on low quality roughage such as standing hay or crop residues, leading to poor performance. Maize is a widely grown cereal and is staple food in most African smallholder systems. It is largely grown and left in the fields to dry to over 80% grain dry matter (DM) before harvesting. Alternatively, maize is popularly harvested at milk stage for roasting, boiling or cooking with vegetables. The stover left after harvesting is potentially a major feed resource to boost ruminant production if properly utilized. However, maize stovers are low in protein or nitrogen (N) concentration (Fadel, 1999) necessitating supplementation with N-rich feed resources. Lucerne (*Medicago sativa* L.) is the predominant pasture legume that has been used as N source for many classes of livestock (Van Keuren & Matches, 1988). However, due to ecological limitations and high agronomic requirements, alternatives to lucerne are sought, of which Sericea lespedeza (lespedeza) has shown potential (Terrill *et al*., 1989). Lespedeza [*Lespedeza cuneata* (Dun-Cours) G. Don] is a tall growing, drought tolerant, coarse-stemmed, non-bloating, deep tap-rooted, self-seeding, perennial legume, adapted to a wide range of soils (Powell *et al*., 2003). It is widely planted in southern USA for grazing, hay and as a soil restoration and conservation crop (Powell *et al*., 2003). Although common lespedeza are known to have a high tannin content which may limit the nutritive value (Turner *et al*., 2005), ruminants such as goats have been reported to tolerate and perform well when consuming high tannin-containing plants (Silanikove *et al*., 1996). Other advantageous features of lespedeza include being long term forage once established (Barkley, 1986), provision of good quality grazing (Schmidt, 1982) and coping well under waterlogged conditions (Guernsey, 1970). In addition, South African farmers have described lespedeza as a low cost pasture, which provides good quality early spring and autumn grazing, makes good quality and 'cheap' hay, hay cures quickly, less wear-and-tear on mowing equipment, increases summer carrying capacity, seldom needs lime and/or phosphorus corrections at planting and does not require the use of pesticides. However, the farmers pointed out disadvantages of lespedeza as slow establishment and inability to be used as foggage ("standing hay") because it is frost sensitive (H. Botha, 2005, Personal communication, Harmonie Trust, P.O. Box 27, Matatiele 4730, South Africa). Lespedeza was imported to South Africa about 15 years ago and commercial seed is now available. Being a relatively new forage in African livestock systems, there is little information on the nutritive value of lespedeza rations with locally available roughages. To provide such information requires screening numerous dietary combinations; ideally through *in vivo* trials. However, *in vivo* trials are expensive and impractical to screen a large number of diets. *In vitro* methods are alternatively used, but the suitability of a chosen method is essential.

In a recent review of the *in vitro* gas production (GP) technique, Dijkstra *et al*. (2005) pointed out that many workers have observed the relationship between degradability predicted from GP using fixed time points and *in vivo* degradability to be only moderate, and that this relationship was improved substantially by including parameters related to the dynamism of GP (Chenost *et al*., 2001; Carro *et al*., 2002). Blümmel & Bullerdick (1997) suggested complementation of *in vitro* GP with residue determination in evaluation of the nutritive value of feeds. In this approach, the residue determination reveals how much substrate is degradable and the gas measurement reflects how much of the degraded portion is converted into fermentation acids and gases. The ratio of substrate truly degraded to gas volume produced, defined as 'partitioning factor' (PF) was found to be valuable in predicting voluntary feed intake. The present study used the *in vitro* gas GP technique to investigate the nutritive effects caused by supplementing locally available roughages in South Africa with lespedeza.

Materials and Methods

Sericea lespedeza re-growth harvested at early (ELP) or late (LLP) flowering stages was used as supplements (SUP). Stovers from the white maize hybrid, PAN 6479, grown in KwaZulu-Natal Province, South Africa and pasture grass hay (GH) were used as roughages (RGH). The maize was either harvested at grain milk (MM) or grain dry (MD) stage. The MM was oven-dried at 50 °C overnight and like the rest of the feeds was milled through 1-mm screen using a Cyclotec mill (Perstorp Analytical Ltd, Bristol, UK). Dry matter was determined by oven drying the samples at 100 °C overnight. Ash was determined by igniting the samples in a muffle furnace at 500 °C overnight. Nitrogen was determined by the micro-Kjeldahl method and crude protein (CP) was calculated as N x 6.25. Methods described by Van Soest *et al*. (1991) were used to determine the neutral detergent fibre (NDF) and acid detergent fibre (ADF). The ADF was further analysed for N content (ADFn). Gross energy (GE) was determined using a DDS isothermal CP500^{TM} bomb calorimeter (Digital Data Systems Ltd., 188 Arbeid Avenue, Johannesburg, SA).

An *in vitro* automated gas production (GP) technique described by Pell & Schofield (1993) was used. A total of 1.0 ± 0.001 g DM in proportions (RGH : SUP) of 0:100, 20:80, 40:60, 20:80 and 100:0 was weighed into 250 mL Duran bottles for *in vitro* incubation. Two sets of incubations were carried out separately, one set with ELP and the other set with LLP rations. In each set, incubation was done thrice with all the treatment rations represented each time. A buffer solution was prepared as described by McDougall (1948), warmed to 39 °C after which 67 mL was added to sample bottles. The bottles, including three sample blanks, were transferred to the incubator maintained at 39 °C where they stayed for 1 h to enable soaking before inoculation. In the meantime a mixture of rumen fluid was collected before morning feeding from three rumen cannulated cows kept on a diet composed of 50:50 grass : lucerne hay. It was filtered through four layers of muslin cloth into a pre-warmed (39 °C) vacuum flask flushed with carbon dioxide (CO_2). Inoculation was done by adding 33 mL of rumen fluid to the sample bottles under stream of CO_2. The bottle lids were tightened and pressure sensors fitted. Settlement time of 30 min was allowed to pass before starting pressure logging at 20 min intervals during a 48-h incubation. Calibration of the pressure logger was done as described by Pell & Schofield (1993). The pressure data was converted to gas volumes using the regression equation and the data fitted into the model described by Campos *et al*. (2004) to determine gas production kinetics as follows:

$$y = \frac{A}{1+\exp\left[2+4a_1(c-t)\right]} + \frac{B}{1+\exp\left[2+4b_1(c-t)\right]}$$

where y is the total gas volume (mL) at time t, A and B the gas volume (mL) from fast (soluble sugars and starch) and slowly (cellulose and hemicellulose) degradable fractions, respectively, a_1 and b_1 are the degradation rates (per h) for fast and slowly degradable fractions, respectively, and c is the time taken (h) for bacteria colonization or lag time (lt).

At the end of incubation, the terminal pH was taken and samples centrifuged at 18 000 g. The supernatant was discarded and the pellet residue (R) dried in a fanned oven at 100 °C for 48 h until constant weight was attained. The difference in mass between R and the mass of the incubated material represented apparent degradability (ApDeg). The residue was refluxed with neutral detergent solution (NDS) and the weight of NDF that resulted, was likewise subtracted from the mass of the incubated material. The value obtained represented the true degradability (TruDeg), as described by Van Soest *et al.* (1991) and Blümmel & Becker (1997). The difference between TruDed and ApDeg was taken to represent microbial matter, as explained by Blümmel *et al.* (1997).

Statistical Analysis System (SAS, 2000) software was used to perform statistical analyses and fitting the model described by Campos *et al.* (2004) to the gas production data. Analysis of variance (ANOVA) was carried out to determine treatment effects whereby stover type, supplement type, supplement level and the interactions among them were used as sources of variation. Means were compared by least significant difference (LSD).

Results

The chemical composition of the feeds is shown in Table 1. The roughages had low CP values that were 48.1, 28.6 and 34.3 g/kg DM for MM, MD and GH, respectively. ELP had markedly higher CP, lower NDF and lower ADF concentrations than LLP. All feeds had similar GE levels with the mean value of 16.5 MJ/kg DM.

Table 1 Chemical composition (g/kg DM) and gross energy (MJ/kg DM) of experimental feeds

Feed	Chemical composition					GE
	CP	NDF	ADF	ADFn	Ash	
Milk stage stover (MM)	48.1	696.1	448.9	1.66	63.8	16.16
Dry stage stover (MD)	28.6	733.3	452.4	1.29	60.2	15.93
Grass hay (GH)	34.3	664.7	463.7	1.46	91.2	16.28
Early flowering stage lespedeza (ELP)	187.4	408.1	282.5	11.7	57.9	17.24
Late flowering stage lespedeza (LLP)	96.8	588.7	436.4	4.79	50.0	16.90

CP - crude protein; NDF – neutral detergent fibre; ADF – acid detergent fibre; ADFn – nitrogen in ADF; GE - gross energy

The pH values at the end of incubation were stable near neutral (6.65 to 6.99) and (6.63 to 7.08) among ELP and LLP rations, respectively, indicating normal fermentation was maintained. The results of degradability, microbial yield, gas production and partitioning factor (PF) of the rations are shown in Table 2 for ELP and Table 3 for LLP rations. Degradability was slightly higher (P < 0.01) in MM as compared to MD and GH (mean 704.9 *vs*. 676.6 and 685.0 g/kg, respectively) among ELP rations. The roughages had similar but lower degradability in LLP rations with mean values of 633.4, 632.6 and 631.1 g/kg for MM, MD and GH rations, respectively. Increasing the proportion of ELP or LLP resulted in decreased (P < 0.001) degradability in all the roughages. Roughages had similar microbial yield in ELP rations, but differed (P < 0.001) in LLP rations whereby GH had the highest value and MD the lowest. Microbial yields were highly variable (cv = 24.7 and 23.9 in ELP and LLP rations, respectively). They were similar in roughages with ELP whereas in LLP rations GH had highest (P < 0.001) values compared to MM and MD (mean 252.6 *vs*. 211.7 and 187.3, respectively). Roughage type affected (P < 0.001) GP with MD having the highest and GH the lowest values. The values ranged from 167.4 to 209.8 and 160.4 to 221.0 mL among ELP and LLP rations, respectively. There was a decrease (P < 0.001) in GP as the proportion of ELP or LLP increased and roughage type x supplement level interaction had an effect (P < 0.05). The stovers showed the same trend in reduction of GP with increase in lespedeza ratio, but for the GH there was an increase in GP from 100:0 to 80:20 RGH : SUP ratio and a decrease as lespedeza ratio increased thereafter. The ratio of degradability to GP, i.e. the partitioning factor (PF) differed (P < 0.01) among the roughages in both ELP and LLP rations but was not affected by supplement level. They ranged from 3.43 to 4.74 and 3.13 to 4.23 in ELP and LLP rations, respectively whereby MD rations had lowest values. These results are detailed in Tables 2 and 3.

Table 2 Mean degradability, microbial yield and gas production of Sericea lespedeza harvested at early flowering stage (ELP), mixed with different roughages stages and fermented using rumen fluid *in vitro*

Roughage type	Roughage : ELP	Degradability g/kg	Microbial yield g/kg	Gas production mL/g	Partitioning factor
Milk stage maize stover	100:0	776.5	173.0	199.1	3.91
	80:20	751.7	135.0	189.5	3.98
	60:40	701.9	199.5	193.4	3.64
	40:60	657.6	264.8	167.4	3.94
	20:80	666.9	185.5	167.6	3.99
	0:100	664.5	236.1	170.8	3.89
Dry stage maize stover	100:0	729.6	150.6	209.8	3.50
	80:20	713.7	140.4	198.9	3.59
	60:40	689.0	193.3	183.6	3.75
	40:60	626.8	166.6	184.7	3.43
	20:80	651.0	226.7	173.9	3.78
Grass hay	100:0	742.6	190.3	204.0	3.65
	80:20	698.4	194.2	176.4	4.74
	60:40	667.5	254.0	176.6	3.79
	40:60	660.7	230.4	173.1	3.82
	20:80	671.3	169.6	172.5	3.92
	LSD	52.90	74.9	24.4	0.66
	P	0.001	0.05	0.001	0.01
	cv	4.9	24.7	8.5	10.9

LSD - least significant difference

The kinetic parameters of gas production are shown in Table 4 for ELP and Table 5 for LLP rations. Gas produced from the soluble fraction (A) was only affected (P < 0.05) by the roughage type whereby GH had the lowest and MM the highest (range 37.4 to 72.3 mL among ELP and 31.1 to 64.0 mL among LLP rations). The rations had similar rates of gas production from the soluble fraction (a_1). The gas produced from the fibre fraction (B) was affected by both roughage type and supplementation level in the ELP rations whereby MM had the lowest value (mean 113.9 mL) as compared to MD and GH, which were similar (mean 129.6 and 129.8 mL, respectively). The rate of gas production from fibre fraction (b_1) was affected by roughage type in ELP rations only with GH having the lowest and MM the highest rate (range 0.023 to 0.026/h). The lt tended to be affected ($P < 0.09$) by supplement level in ELP rations (range 1.83 – 6.59 h) whereby it decreased as the proportion of ELP increased. In LLP rations, roughage type, supplement level and supplement level x roughage type interaction all had effects ($P < 0.001$) on lt (range 0.88 to 9.61 h). Among the roughages, MM had the shortest and GH the highest lt. The lt shortened as the supplementation level increased across all roughages. The interaction between roughage type and supplement level is depicted in Figure 1.

Table 3 Mean degradability, microbial yield and gas production of Sericea lespedeza harvested at late flowering stage (LLP), mixed with different roughages stages and fermented using rumen fluid *in vitro*

Roughage type	Roughage : LLP	Degradability g/kg	Microbial yield g/kg	Gas production mL/g	Partitioning factor
Milk stage maize stover	100:0	709.5	229.5	205.4	3.50
	80:20	667.8	214.1	199.4	3.35
	60:40	645.9	184.7	186.2	3.47
	40:60	607.7	223.9	171.8	3.55
	20:80	562.0	212.0	166.7	3.37
	0:100	533.5	252.3	148.2	3.61
Dry stage maize stover	100:0	742.5	185.8	221.0	3.36
	80:20	679.0	225.7	216.6	3.13
	60:40	599.7	143.4	186.7	3.21
	40:60	611.4	216.4	181.6	3.38
	20:80	565.7	171.7	163.8	3.47
	0:100	533.5	252.3	148.2	3.61
Grass hay	100:0	719.2	295.9	172.5	4.23
	80:20	659.5	243.3	190.4	3.47
	60:40	627.9	241.7	174.8	3.62
	40:60	596.2	228.7	171.9	3.47
	20:80	578.5	261.0	160.4	3.62
	0:100	533.5	252.3	148.2	3.61
	LSD	45.37	77.17	17.12	0.45
	P	0.001	0.001	0.001	0.01
	cv	4.8	23.9	6.3	8.6

LSD - least significant difference

Discussion

The maize stover exhibited high nutritive potential, especially as reflected from the highest degradability values where there was no supplementation. Although it was anticipated that there would be substantial decline in nutritive value of MD as compared to MM, the result indicated the decline was minimal. Similar observations have been made by Akbar *et al*. (2002) where 72 h degradability of early harvested stovers ranged from 70.1 – 76.8% and those of late harvested ranged from 69.4 to 74.6% in several maize varieties. The present findings thus support the observation of Sutton *et al*. (1999) where small and non-significant decrease in whole tract digestibility by dairy cows fed maize silage of different maturity was observed. Sheep fed maize stover supplemented with various multipurpose trees containing tannins and other antinutritive compounds had degradability ranging from 490 to 518 g/kg (Hindrichsen *et al*., 2004).

Table 4 Mean gas production parameters[1] of Sericea lespedeza harvested at early flowering stage (ELP), mixed with different roughages stages and fermented using rumen fluid *in vitro*

Roughage type	Roughage : ELP	A	B	a_1	b_1	lt
Milk stage maize stover	100:0	57.7	133.6	0.152	0.029	4.88
	80:20	72.3	114.9	0.123	0.027	3.48
	60:40	66.4	119.7	0.097	0.025	1.83
	40:60	53.5	106.8	0.104	0.026	3.15
	20:80	57.2	97.3	0.135	0.025	3.47
	0:100	54.1	110.6	0.105	0.026	2.97
Dry stage maize stover	100:0	57.4	145.1	0.103	0.024	4.61
	80:20	58.3	132.5	0.128	0.025	4.83
	60:40	48.0	128.6	0.129	0.025	5.02
	40:60	38.6	141.4	0.094	0.023	2.89
	20:80	53.5	107.1	0.150	0.025	4.03
	0:100	54.1	110.6	0.105	0.026	2.97
Grass hay	100:0	39.8	153.9	0.140	0.024	6.59
	80:20	46.3	108.6	0.137	0.025	5.82
	60:40	37.4	131.4	0.150	0.024	5.30
	40:60	39.8	126.9	0.101	0.024	3.34
	20:80	42.5	130.4	0.52	0.024	2.35
	0:100	54.1	110.6	0.105	0.026	2.97
	LSD	27.05	30.14	0.177	0.005	3.03
	P	0.01	0.01	ns	0.05	ns
	cv	29.0	13.4	69.7	10.3	41.1

[1] Based on equation by Campos *et al.* (2004) where A and B are the gas volume (mL) from fast (soluble) and slowly (fibre) degradable fractions, respectively; a_1 and b_1 are the degradation rates (per h) of soluble and fibre fractions, respectively; lt is lag time
LSD - least significant difference

Although these values are lower than those of the current study, they are reflective of the lower degradability often observed *in vivo* compared to *in vitro*. Flachowsky *et al.* (1991) showed that cultivars with higher straw quality were not consistently associated with lower grain yield, and this gives an opportunity to develop or select a variety with high quality crop residue without sacrificing grain yield. As for the GH used, its CP concentration was within the range of 23.3 – 50.9 g/kg, reported by Ali *et al.* (2001) in several tropical grasses. These values are fairly low compared to those reported by Aganga & Tshwenyane (2004) ranging from 53 to 126 g/kg of a tropical grass. Hence, this emphasizes the role of proper management to maintain the quality of grass pastures and/or supplementation with CP or nitrogen sources where these poor quality roughages must be utilized. The importance of a forage such as lespedeza stems from this. Muyekho *et al.* (2003) found that farmers considered factors relating directly to the animal as more important than factors relating to the agronomic characteristics of the forage. These included palatability and potential to increase production. Agronomic factors valued by the farmers included high yields, drought tolerance and diseases/pests resistance. Their results also suggested that farmers were reluctant to adopt forage crops that require planting on annual basis. Other criteria that needed to be considered include factors that may cause direct harm to the livestock, e.g. feeding of lucerne was reported to have the possibility of causing bloat. These observations closely corroborate those of a farmer in KwaZulu-Natal in preferring lespedeza forage. The farmer who had endeavoured for 30 years to find a suitable forage described lespedeza as a 'miracle' pasture that can produce cost-effective grazing and hay on a sustainable basis (H. Botha, 2005, Personal communication, Harmonie Trust, P.O. Box 27, Matatiele 4730, South Africa). Often scientists ignore many of these factors in early stages of species/variety development and yet inclusion of farmers' criteria could minimize the costs of research and enhance the uptake of technology. This concern has also been pointed out by Muyekho *et al.* (2003).

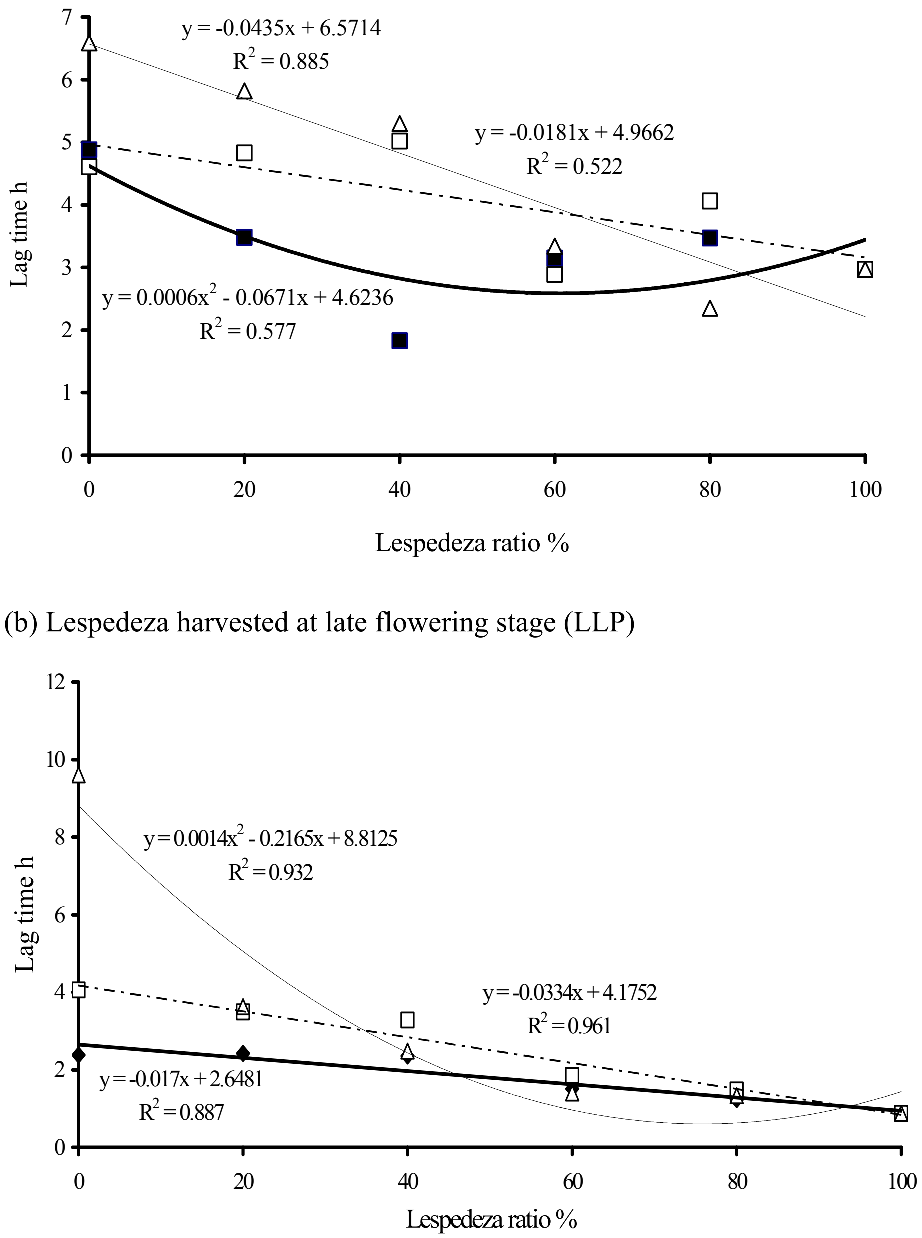
Table 5 Mean gas production parameters[1] of Sericea lespedeza harvested at late flowering stage (LLP), mixed with different roughages stages and fermented using rumen fluid *in vitro*

Roughage type	Roughage : LLP	A	B	a_1	b_1	lt
Milk stage maize stover	100:0	64.0	135.9	0.096	0.026	2.38
	80:20	59.2	133.8	0.067	0.023	2.42
	60:40	67.4	110.9	0.099	0.025	2.36
	40:60	61.0	101.3	0.101	0.025	1.51
	20:80	58.2	102.3	0.089	0.025	1.23
	0:100	66.1	75.7	0.096	0.027	0.88
Dry stage maize stover	100:0	60.4	153.0	0.105	0.024	4.06
	80:20	61.2	147.8	0.088	0.025	3.49
	60:40	56.7	122.7	0.103	0.025	3.28
	40:60	66.4	107.6	0.098	0.025	1.85
	20:80	53.6	102.9	0.078	0.024	1.48
	0:100	66.1	75.7	0.096	0.027	0.88
Grass hay	100:0	31.1	154.3	0.087	0.024	9.61
	80:20	56.4	126.7	0.097	0.024	3.63
	60:40	60.0	106.8	0.090	0.023	2.48
	40:60	51.1	113.9	0.076	0.023	1.40
	20:80	51.1	100.3	0.083	0.023	1.33
	0:100	66.1	75.7	0.096	0.027	0.88
	LSD	21.8	29.0	0.14	0.003	1.86
	P	0.05	0.001	ns	ns	0.001
	cv	19.9	15.1	85.0	7.7	37.5

[1] Based on equation by Campos *et al.* (2004) where A and B are the gas volume (mL) from fast (soluble) and slowly (fibre) degradable fractions, respectively; a_1 and b_1 are the degradation rates (per h) of soluble and fibre fractions, respectively; lt is lag time
LSD - least significant difference

The reduction in degradability and gas production as the lespedeza ratio increased can be attributed to its chemical composition, particularly the tannin content. Although tannin content was not determined in the current study, Turner *et al.* (2005) observed high tannin concentration of 23.1 mg/g in lespedeza, and a much lower value of 0.24 mg/g in lucerne. Similar to the present findings, the presence of tannins in browses depressed the *in vitro* gas and SCFA production (Getachew *et al.*, 2000). The depression in the fermentation could be a result of either direct interaction between tannins and the bacterial cell wall (Jones *et al.*, 1994) or the effect of tannin on microbial enzymes (Bae *et al.*, 1993; Ngwa *et al.*, 2003), with the net effect of hampering microbial growth as reported by Field & Lettinga (1992). Getachew *et al.* (2000) showed that the inhibitory effect of tannin can be removed by adding polyethylene glycol (PEG), implying that a solution to this anti-nutritive factor is available. On the other hand, it is noteworthy that both advantageous and negative effects of tannin containing feeds have been reported from *in vivo* findings. Lespedeza offered to sheep had low digestibility due to high tannin concentration (Terrill *et al.*, 1989). Condensed tannin (CT) containing forages had slower rates of digestion in the rumen, but greater ruminal escape values (Albrecht & Broderick, 1990). Kaitho *et al.* (1993) speculated that sheep fed high-tannin legumes had an overall shortage of rumen-degradable-N resulting in impaired fibre digestibility and reduced weight gain. On the positive

(a) Lespedeza harvested at early flowering stage (ELP)



(b) Lespedeza harvested at late flowering stage (LLP)

Figure 1 Effect of increasing ratio of lespedeza on lag time of milk stage (—■—), dry stage (–·–□–·–) maize stover and grass hay (—Δ—) rations fermented *in vitro*

side, Caygill & Mueller-Harvey (1999) stated that some tanniniferous feeds can produce beneficial effects in ruminants, e.g. improved amino acid absorption and anthelmintic effects. Douglas *et al.* (1995) reported greater live weight gain when lambs grazed birdsfoot trefoil *vs.* lucerne, which was attributed to the influence of CT in the ruminant digestive tract. Medium concentrations of CT (45 to 55 g CT/kg DM) in forages can improve N-use efficiency in ruminants (Min *et al.*, 2003).

Efficiency of rumen microbial protein synthesis is a key factor in the protein evaluation systems for ruminants. However, accurate determination of microbial yield *in vitro* is still an elusive aspect of ruminant nutrition. This is because of several limitations of the batch culture applied in the *in vitro* systems (Dijkstra *et* *al*., 1998, Rymer *et al*., 2001). In the current work, the two main issues causing difficulty in making inference to microbial yield values are the duration of incubation and possibility that tannin-protein complexes were formed (Haggerman & Robbins, 1987) and were washed away by NDS, hence increasing the values estimated. Blümmel *et al*. (2003) found that PF at the time of the estimated peak microbial production *in vitro*, but not after 16 or 24 °h of incubation, was well correlated with observed microbial efficiency *in vivo*. Rymer *et al*. (2001) found the time factor to play important role with regard to the fermentation products stoichiometry and microbial recycling during incubation. They found good relationships between predicted and observed gas volumes at early hours (8 h), which was lost by 48 h. Thus the present 72 h incubation could therefore be regarded as far too long, but was essential in assessing maximal degradability. Nevertheless, from PF values it can be deduced that GH was superior to the stovers as it had higher values with both ELP and LLP rations.

From the GP kinetics, the higher A value of MM depicts higher availability of soluble and readily fermentable compounds at the early stover stage and this is an indication of superior nutritive quality over MD in particular. In ruminant nutrition, the fermentation of fibre component depicted by B and the rapidity by which this happens are crucial. Krishnamoorthy *et al*. (1995) pointed out that information on lt and rate of fermentation as indices of rapidity with which organic matter is fermented in the rumen can be useful in formulating diet with a desirable rate of fermentation. From the current results it appears that supplementing the roughages with lespedeza induces the positive impact by reducing the lt, the greatest being with GH. Two factors can be inclusively stipulated to be responsible for the shorter lt of stovers. Firstly, the fact that unsupplemented stovers had higher A values as compared to GH indicated that the former had higher content of soluble fraction, which boosted microbial colonization. Secondly, grinding the much coarser stovers through 1 mm screen resulted in finer material as compared to GH; implying the stovers had higher surface area exposed for colonization. Both of these factors would cause reduction in lag. It appeared that fibre degradation was optimal at the RGH : SUP ratio of 60:40 and 40:60 for in ELP rations with MM and MD, respectively, when B was maximal and lt minimal. For GH rations, the pattern was not as clear although 40:60 rations could be recommended as they had modest values of B and lt. Among the LLP rations, it was interesting that the three roughages had nearly the same lt at RGH : SUP ratio of 40:60, which was near the inflection point for GH rations (Figure 1). Perhaps this can be regarded as a suitable supplementation level given that B remained nearly constant as the level of LLP increased beyond this point. The other parameters (a_1 and b_1) did not conspicuously reflect the impacts of supplementation within roughage types, hence were difficult to be used in making inferences.

Conclusions

Although the roughages were of poor quality reflected in low CP and high fibre concentrations, they showed high potential due to high degradability. The maturity stage influenced the nutritive characteristics of the forages whereby depreciation in quality with age was observed in both maize stovers and lespedeza, but the effect was small in the stovers, particularly with regard to degradability. The positive impacts arising from supplementation with lespedeza included enhancing fermentation of fibre fraction and decreasing rumen microbial colonization lag time. It was apparent that there is a limit to which lespedeza can be added to optimise the positive impacts it induces. The results indicate that when using roughages with similar quality as those studied, the lespedeza ratio of 40-60% of DM consumed can be beneficial. This needs ratification through *in vivo* trials.

Acknowledgements

Financial support for this work was provided by the South Africa National Research Foundation (NRF). Technical support was provided by S. van Malsen, D. Davies, M. Hundley, S.M. Opperman, S.B. Opperman and S. Khumalo; and logistical support by R.M. Gous and M. Bowen. The development of this article evolved from a Scientific writing workshop conducted by J. Kerchhoff and M. Birgham (UKZN-Language Centre). J.O. Ouda's study was sponsored by the Kenya Agricultural Research Institute. These contributions are highly appreciated.

References

Aganga, A.A. & Tshwenyane, S., 2004. Potentials of guinea grass (Panicum maximum) as forage crop in livestock production. Pakistan J. Nutr. 3 (1), 1–4.

Akbar, M.A., Lebzien, P. & Flachowsky, G., 2002. Measurement of yield and *in situ* dry matter degradability of maize varieties harvested at two stages of maturity in sheep. Anim. Feed Sci. Technol. 100, 53-70.

Albrecht, K.A. & Broderick, G.A., 1990. Degradation of forage legume protein by rumen microorganism. In: Agronomy Abstract. American Society of Agronomy, Madison, W.I. p.185.

Ali, M., Qamar, I.A., Ali, A, Arshad, M. & Iqbal, J., 2001. Evaluation of tropical grasses for forage yield and crude protein content in the Pothwar Plateau of Pakistan. J. Biol. Sci. 1, 466-467.

Bae, H.D., McAllister, T.A., Yanke, J., Chen, K.J. & Muir, A.D., 1993. Effect of condensed tannins on endogluconase activity and filter paper digestion by *Fibrobacter succinogenes* S85. Appl. Environ. Microbiol. 59, 2132-2138.

Barkley, T.M., 1986. Flora of the Great Plains. Great Plains Flora Assoc., Univ. Press of Kansas, Kansas, USA. 1392 pp.

Blümmel, M. & Becker, K., 1997. The degradability characteristics of fifty-four roughages and roughage neutral fibre as described by *in vitro* gas production and their relationship to voluntary feed intake. Br. J. Nutr. 77, 757-768.

Blümmel, M. & Bullerdick, P., 1997. The need to complement *in vitro* gas measurements with residue determination from *in sacco* degradabilities to improve the prediction of voluntary in take of hays. Anim. Sci. 64, 71-75.

Blümmel, M., Karsli, A. & Russel, J.R., 2003. Influence of diet on growth yields of rumen microorganisms *in vitro* and *in vivo*: influence on growth yield of variable carbon fluxes to fermentation products. Br. J. Nutr. 90, 625-634.

Blümmel, M., Steinggaβk, H. & Becker, K., 1997. The relationship between *in vitro* gas production, *in vitro* microbial biomass yield and 15N incorporation and its implications for prediction of voluntary feed intake of roughages. Br. J. Nutr. 77, 911-921.

Campos, F.P, Sampaio, A.A.M., Bose, M.L.V., Vieira, P.F. & Sarmento, P., 2004. Evaluation of *in vitro* gas production of roughages and their mixtures using curves subtraction method. Anim. Feed Sci. Technol. 116, 161-172.

Carro, M.D., López, S., González, J.S., Ovejero, F.J. & Ranilla, M.J., 2002. *In vitro* methods as predictors of voluntary intake and digestibility of hays fed to sheep. Aust. J. Agric. Res. 53, 471–479.

Caygill, J.C. & Mueller-Harvey, I., 1999. Secondary Plant Products - Considerations for Animal Feeds. Nottingham University Press, Nottingham, UK. 129 p.

Chenost, M., Aufrere, J. & Macheboeuf, D., 2001. The gas-test technique as a tool for predicting the energetic value of forage plants. Anim. Res. 50, 349–364.

Dijkstra, J., France, J. & Davies, D.R., 1998. Different mathematical approaches to estimating microbial protein supply in ruminants. J. Dairy Sci. 81, 3370–3384.

Dijkstra, J., Kebreab, E., Bannink, A., France, J. & Lopez, S., 2005. Application of the gas production technique to feed evaluation systems for ruminants. Anim. Feed Sci. Technol. 123, 561-578.

Douglas, G.B., Wang, Y., Waghorn, G.C., Berry, T.N., Purchas, R.W., Foote, A.G. & Wilson, G.F., 1995. Liveweight gain and wool production of sheep grazing *Lotus corniculatus* and lucerne (*Medicago sativa*). N. Z. J. Agric. Res. 38, 95-104.

Fadel, J.G., 1999. Quantitative analyses of selected plant by-product feedstuffs, a global perspective. Anim. Feed Sci. Technol. 79, 255-268.

Field, J.A. & Lettinga, G., 1992. Toxicity of tannic compounds to microorganisms. In: Plant Polyphenols: Synthesis, Properties, Significance. Eds. Hemingway, R.W. & Laks, E., Pleneum Press, New York. pp. 673-692.

Flachowsky, G., Tiroke, K. & Schein, G., 1991. Botanical fractions of straw of 51 cereal varieties and *in sacco* degradability of various fractions. Anim. Feed Sci. Technol. 34, 279–289.

Getachew, G., Makkar, H.P.S. & Becker, K., 2000. Tannins in tropical browses: Effects on *in vitro* microbial fermentation and microbial protein synthesis in media containing different amounts of nitrogen. J. Agric. Food Chem. 48, 3581-3588.

Guernsey, W.J., 1970. Sericea lespedeza, its use and management. U.S.D.A. Farmers Bull. 2245, 1-29.
Haggerman, A.E. & Robbins, C., 1987. Implications of soluble protein tannin complexes for tannin analysis and defence mechanisms. J. Chem. Ecol. 13, 1243-1259.
Hindrichsen, I.K., Osuji, P.O., Odenyo, A.A., Madsen, J. & Hvelplund, T., 2004. Effect of supplementation of maize stover with foliage of various tropical multipurpose trees and Lablab purpureus on intake, rumen fermentation, digesta kinetics and microbial protein. Anim. Feed Sci. Technol. 113, 83-96.
Jones, G.A., McAllister, T.A., Muir, A.D. & Cheng, K.J., 1994. Effect of sainfoin (Onobrychis viciifolia Scop.) condensed tannins on growth and proteolysis by four strains of ruminant bacteria. Appl. Envir. Microbiol. 60, 1374-1378.
Kaitho, R.J., Tamminga, S. & Bruchen, J., 1993. Rumen degradation and *in vivo* digestibility of dried Calliandra calothysus leaves. Anim. Feed Sci. Technol. 43, 19-30.
Krishnamoorthy, U., Soller, H., Steingass, H. & Menke, K.H., 1995. Energy and protein evaluation of tropical feedstuffs for whole tract and ruminal digestion by chemical analyses and rumen inoculum studies *in vitro*. Anim. Feed Sci. Technol. 52, 177-188.
McDougall, E.I., 1948. Studies on ruminant saliva. The compostion and output of sheep saliva. Biochem. 43, 99.
Min, B.R., Barry, T.N., Attwood, G.T. & McNabb, W.C., 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. Anim. Feed Sci. Technol. 106, 3–19.
Muyekho, F.N., Mose, L. & Cheruiyot, D.T., 2003. Development and transfer of forage production technologies for smallholder dairying: case studies of participatory eveluation of species and methods of establishment in Western Kenya. Trop. Grassl. 37, 251-256.
Ngwa, A.T., Nsahlai, I.V. & Iji, P.A., 2003. Effect of feeding legume pods or alfalfa in combination with poor quality grass straw on microbial enzyme activity and production of VFA in the rumen of South African Merino sheep. Small Rumin. Res. 48, 83-94.
Pell, A.N. & Schofield, P., 1993. Computerized monitoring of gas production to measure forage digestion *in vitro*. J. Dairy Sci. 76, 1063-1073.
Powell, M.C., Muntifering, R.B., Lin, J.C. & Chappelka, A.H., 2003. Yield and nutritive quality of sericea lespedeza (*Lespedeza cuneata*) and little bluestem (*Schizachyrium scoparium*) exposed to ground-level ozone. Environ. Poll. 122, 313-322.
Rymer, C., Fakhri, S., Moss, A.R. & Givens, D.I., 2001. Relationship between the production of short chain fatty acids and gas when proteins are incubated *in vitro*. Br. Soc. Anim. Sci. p. 134.
SAS, 2000. Statistical Analysis System user's guide (Version 8). SAS Institute Inc., SAS Campus Drive, Cary, N.C., USA.
Schmidt, S.P., Donnelly, E.D., Hoveland, C.S. & Moore, R.A., 1982. Steers make good gains grazing sericea lespedeza and alfalfa. Highlights of Agricultural Research, Auburn Univ. Agric. Exp. Sta. 29(4), 4.
Silanikove, N., Gilboa, N., Perevolotsky, Z. & Nitsan, Z., 1996. Goats fed tannin containing leaves do not exhibit toxic syndromes. Small Rumin. Res. 21, 195-201.
Sutton, J.D., Cammaell, S.B., Phipps, R.H., Beever, D.E. & Humphries, D.J., 1999. The effect of maize silage maturity on digestibility and energy balance of dairy cows. Br. Soc. Anim. Sci. p. 3.
Terrill, T.H., Windham, W.R., Hoveland, C.S. & Amon, H.E., 1989. Forage preservation method influences on tannin concentration, intake and digestibility of sericea lespedeza by sheep. Agron. J. 81, 435-439.
Tilley, J.M.A. & Terry, R.A., 1963. A two-stage technique for the digestion of forage crops. J. Br. Grassl. Soc. 18, 104-111.
Turner, K.E., Wildeus, S. & Collins, J.R., 2005. Intake, performance, and blood parameters in young goats offered high forage diets of lespedeza or alfalfa hay. Small Rumin. Res. 59, 15-23.
Van Keuren, R.W. & Matches, A.G., 1988. Pasture production and utilization. Agron. 29, 515-538.
Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.